formed remotely using vendor-specific hardware and software. In this study, we examined the technical feasibility of displaying diagnostic quality images on a remote site PC with an Internet WEB browser (Netscape 2.02). Blinded review of 26 consecutive studies established an interpretive concordance of 83% for any abnormality, 81% for multivessel ischemia, and 85% for territory of abnormality when compared with images read from films. Technical issues involved were: 1) Development of a display format for presentation of axial slices, polar maps with quantitative information, and planar images on a 1020 × 820, 8 bit display; 2) Security of the WEB site; 3) Image conversion from raw data to 8 bit grey scale (GIF image format); 4) Average download time of 104 ± 8 seconds using a 28.8 kbs modem with V34 compression; 5) Display on a Pentium 100 with a 1 Mb video card; 6) Video card display settings of 8 bit color and 800 × 600 resolution. Image quality scored by two independent, expert observers on a scale of 1–5 (1 = inadequate; 2 = poor; 3 = adequate; 4 = good; 5 = superior) ranged from 3 to 4 (average value = 3.7).

Conclusion: This investigation establishes the technical feasibility of interpretation of images over the Internet using a PC and a WEB browser. While the current configuration permits reasonably high interpretive concordance relative to traditional films, incorporation of additional data (such as rotating projection images and pertinent aspects of a patient's history) and a higher quality image display medium may further improve results.

1074 Cardiovascular Molecular Biology: Neurohormones and Vasoactive Modifiers

Wednesday, March 19, 1997, Noon–2:00 p.m.
Anaheim Convention Center, Hall E
Presentation Hour: 1:00 p.m.–2:00 p.m.

1074-118 Design, Synthesis and In Vivo Actions of a Novel Chimeric Peptide to B-Type (BNP) and C-Type (CNP) Natriuretic Peptides

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CNP, a member of the natriuretic peptide system (NPS) of endothelial origin, is a potent venodilator with minimal natriuretic actions. CNP exerts vascular actions via generation of cGMP after binding to the NPR-B receptor. BNP, a member of the NPS of myocardial origin, is the most potent natriuretic peptide. BNP mediates its natriuretic effect via the NPR-A receptor. Both CNP and BNP possess a 17-amino acid ring structure, but BNP has a 6-amino acid COOH-terminus which CNP lacks. This study was undertaken to investigate the in vivo actions of a novel 28-amino acid chimeric peptide termed "CB-NP". This peptide possesses the 22-amino acid structure of CNP and the 6-amino acid COOH terminus of BNP. CB-NP was synthesized using FLMC chemistry on a peptide synthesizer and purified by reverse phase HPLC. In 5 anesthetized dogs, cardiorenal function was measured in response to CB-NP (50 ng/kg/min IV) followed by co-infusion of HS-142-1, a dual inhibitor of NPR-A and B receptors (3 mg/kg IV bolus). CB-NP decreased right atrial pressure (−1.2 ± 0.3 to −2.2 ± 0.4 mmHg, $p < 0.05$) and cardiac output (3.6 ± 0.2 to 3.1 ± 0.3 l/min, $p < 0.05$) consistent with a decrease cardiac preload. HS-142-1 did not reverse these effects. CB-NP was markedly natriuretic with increases in glomerular filtration rate (38 ± 4 to 52 ± 5 ml/min, $p < 0.05$), urinary sodium excretion (65 ± 24 to 240 ± 78 mEq/min, $p < 0.05$), fractional sodium excretion (1.1 ± 0.4 to 3.3 ± 1.0%, $p < 0.05$) and a decrease in distal fractional reabsorption of sodium (96.4 ± 1.0 to 91.2 ± 1.8%, $p < 0.05$). CB-NP increased plasma cGMP (3.1 ± 0.9 to 7.1 ± 2.8 pmol/ml, $p < 0.05$) and urinary cGMP excretion (733 ± 124 to 1843 ± 356 pmol/ml, $p < 0.05$). HS-142-1 abolished all these effects. The current study suggests that CB-NP possesses the venodilating actions of CNP and the natriuretic actions of BNP which are secondary to unique co-activation of both NPR-A and B receptors. This unique peptide may have therapeutic potential in cardiorenal disease states.

1074-119 Control of Cardiac Hypertrophy and Natriuretic Peptide Production by Endogenous Angiotensin II in Experimental Heart Failure

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Angiotensin II (ANG II) induces cell hypertrophy and natriuretic peptide (NP) production supporting a role for atrial (ANP) and brain (BNP) natriuretic peptides as serum markers for left ventricular hypertrophy (LVH). The effect of chronic ACE inhibition (ACEI) in congestive heart failure (CHF) upon LVH and plasma NPs remains poorly defined. We tested the hypothesis that inhibition of ANG II with ACEI during the progression of CHF would prevent LVH and attenuate increases in circulating ANP and BNP, and that these NPs would be powerful serum markers for LVH with and without ACEI during CHF. CHF was produced by rapid ventricular pacing in the presence (n = 6, CHF + ACEI) and absence (n = 8, CHF) of enalapril (10 mg po bid). Plasma NPs and ANG II were determined during the progression of CHF. Left ventricular mass index (LVMI) was determined at autopsy. ACEI prevented the late increase in ANG II associated with CHF (Δ 10 ± 3 vs 143 ± 67, $P < 0.05$), and attenuated the increase in ANP and BNP during the progression of CHF. without differences in cardiac filling pressures. * denotes $p < 0.05$ vs CHF

	CHF			CHF + ACEI		
	BL	Early	Late	BL	Early	Late
ANP (pg/ml)	43 ± 11	233 ± 64	416 ± 93	50 ± 10	140 ± 41	235 ± 39*
BNP (pg/ml)	19 ± 6	71 ± 20	155 ± 32	20 ± 6	20 ± 7*	54 ± 7*

ACEI prevented the increase in LVMI (4.2 ± 0.1 vs 4.9 ± 0.2 mg/kg TBW, $p < 0.05$) and ANP and BNP correlated with LVMI ($r = 0.65$, $p < 0.01$ and $r = 0.87$, $p < 0.001$, respectively). We conclude that endogenous ANG II modulates LVH and the activation of circulating NPs in experimental CHF. Additionally, ACEI prevents LVH and activation NPs in experimental CHF, and both ANP and BNP are powerful markers of LVH in the presence and absence of ACEI.

1074-120 Quantitative Expression of the Tissue Renin Angiotensin System in Left Ventricles with Aortic Stenosis Compared to Normal Hearts

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Angiotensin II is a potent growth factor in cardiovascular tissues. It's local effects are determined by the supply with angiotensinogen, which is converted via ACE and chymase to the biological active compound. Most of it's known effects are mediated by the AT1-receptor. The AT2-receptor is mainly expressed in fetal tissues and wound healing, indicating it's predominant role in growth, development and differentiation.

We investigated the expression of the tissue renin angiotensin system (tissue RAS) in tissue samples from left ventricles with aortic stenosis (n = 12) compared to normal left ventricles from not transplanted donor hearts (n = 6) by quantitative reverse transcription-polymerase chain reaction (RT-PCR). Recently, we described the construction of a multistandard cRNA, which enables the quantification of the gene expression of the different components of the human tissue RAS by coamplification with specific oligonucleotide primers.

Results: the AT1-receptor expression decreased markedly in left ventricles with aortic stenosis ($p < 0.01$), while there was no significant difference in the AT2-receptor expression. The angiotensinogen expression is significantly enhanced compared to normal hearts. No significant differences were found in the expression of ACE and chymase.

Conclusion: The study revealed a different regulation of the AT-receptor subtypes in left ventricular hypertrophy caused by aortic stenosis, which is associated by an enhanced expression of the angiotensin II precursor angiotensinogen.

1074-121 Strong Positive and Negative cis-acting Elements Control Endothelin B Receptor Expression

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The Endothelin B receptor (ET_B) is expressed in neural and endothelial cells. It plays an important role in the development of the neural crest and is coupled to the production of factors involved in normal endothelial functions such as nitric oxide. Its absence or downregulation likely plays a key role in the development of pulmonary hypertension and other instances of endothelial dysfunction. To understand its regulation, we obtained mouse ET_B genomic clones and characterized a 1.6 kb fragment that contained all of exon I - including 0.2 kb of 5′ untranslated region (UTR)- and approximately 0.66 kb of the 5′ flanking region. The transcription initiation site is located at −200 relative to the translational start site. Transfection experiments showed that this fragment functioned as a specific promoter of a luciferase reporter gene in P19 neural cells expressing ET_B. Serial deletions between −660 to −180 led to a > 12-fold increase in luciferase activity. The deletion of −180 to −75 (including a 40 bp long region of 83% homology between the mouse and the human genes) resulted in a 95% loss of activity of the reporter gene. In conclusion: 1) strong silencer elements are present in the 5′ flanking region of

the ET_B gene, 2) A highly conserved region in its 5'UTR harbors an element responsible for high level expression of a reporter gene in cells expressing ET_B. These elements may help us understand ET_B regulation during neural crest development and in conditions associated with endothelial dysfunction such as heart failure, pulmonary hypertension and atherosclerosis.

1074-122 Aldosterone synthase gene polymorphism predicts left ventricular size and function in persons free of heart disease

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Aldosterone has many effects on heart and circulation. Genetic variation in aldosterone synthesis could therefore have cardiac implications. Aldosterone synthase (CYP11B2) catalyzes the last steps of aldosterone formation. We studied whether CYP11B2 gene polymorphism predicts left ventricular (LV) size, mass or function in humans.

84 persons (40 men) free of heart disease born in 1954 were studied by M-mode and Doppler echocardiography and genotyped by polymerase chain reaction for −344 cytosine/thymidine (C/T) polymorphism in the promoter of the CYP11B2 gene. The LV measurements (mean ± SD) by the C/T genotype group were as follows:

Measurement	−344TT n = 22	−344CT n = 42	−344CC n = 20	P (anova)
EDD, mm	47 ± 3	50 ± 4	51 ± 4	0.002
ESD, mm	31 ± 3	34 ± 4	35 ± 6	0.013
Mass, g	149 ± 39	169 ± 46	182 ± 58	0.082
E/A	1.7 ± 0.3	1.5 ± 0.3	1.6 ± 0.3	0.024
AFF, %	23 ± 6	27 ± 6	26 ± 6	0.004

EDD = end-diastolic diameter; ESD = end-systolic diameter; E/A = transmitral early/atrial velocity ratio; AFF = atrial filling fraction. The influence of CYP11B2 genotype on LV measurements was independent of sex, body size, blood pressure and salt intake.

We conclude that DNA polymorphism in the CYP11B2 gene predicts LV structure and function in persons free of heart disease.

1074-123 Effect of Estrogen on Protein and DNA Synthesis and Estrogen Receptor Status in Cardiac Fibroblasts

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Estrogen exerts a clinically relevant anti-atherogenic effect in women. In addition, estrogen may bind to receptors on vascular smooth muscle cells and modulate the growth of these cells. Little, however, is known about its direct effects on cardiac fibroblasts, which are responsible for extracellular matrix production in the heart. We, therefore, investigated the role of estrogen on male adult rabbit cardiac fibroblasts, which were grown until subconfluent in DMEM supplemented with 10% FBS. Western immuno slot blot analysis demonstrated that cardiac fibroblasts express estrogen receptor protein. The density of the estrogen receptor significantly increased with the degree of confluency from 24 through 120 hours in culture (685%, $p = 0.005$). Next, we investigated the effect of estrogen on DNA synthesis measuring ^{3}H-thymidine incorporation. Estrogen dose-dependently decreased DNA synthesis of cardiac fibroblasts. The maximum decrease was observed with 10 nM estrogen (78%, $p = 0.0005$). In addition, estrogen treatment decreased protein synthesis, as measured by ^{3}H-phenylalanine incorporation by 84% ($p < 0.0001$). In contrast, transforming growth factor beta 1 (TGF-β_1) protein concentrations increased by 178% ($p < 0.05$). Furthermore, treatment with 10 nM, 500 nM, and 10 μM exogenous estrogen further increased estrogen receptor protein immunoreactivity by 13% ($p < 0.0005$), 17% ($p < 0.005$), and 18% ($p < 0.005$), respectively. We, therefore, conclude that estrogens modulate protein and DNA synthesis in cardiac fibroblasts. The anti-proliferative effects of estrogen may involve the induction of TGF-β_1 and/or a positive feedback on the expression of its receptor.

1074-124 Interactions Between Estradiol and Mechanical Strain in Human Vascular Smooth Muscle Cells in Culture

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The cellular basis of the cardioprotective effects of estrogen are largely unknown. A direct inhibitory effect on vascular smooth muscle growth has been proposed, but conflicting data exist, showing stimulation of mitogenesis under some circumstances. We examined the effect of different concentrations of 17β-estradiol on mitogenesis induced by cyclic mechanical strain in human vascular smooth muscle cells in culture. Cells were grown to confluence on fibronectin-coated plates with silicone-elastomer bottoms. They were then exposed to cyclic mechanical strain (60 cycles/min, 48 h), in the presence and absence of 17β-estradiol (1 nM or 1 μM). 3H-Thymidine was measured during the last six hours. Cyclic mechanical strain induced 1.5 to 2 fold increases in DNA synthesis. While estradiol, 1 nM caused an inhibition of strain-induced mitogenesis, estradiol 1 μM caused an enhancement. Thymidine incorporation data (cpm/well) are shown below.

Control	3934 ± 388
Strain	6753 ± 388
Strain + Estradiol 1 nM	3397 ± 465
Strain + Estradiol 1 μM	8875 ± 515

The extent of inhibition of strain-induced mitogenesis by physiological concentrations of estradiol (1 nM) was attenuated by the estrogen receptor antagonist ICI 182,780 (1 nM). We conclude that estrogen, in physiological concentrations, inhibits strain-induced mitogenesis via an estrogen-receptor mediated process, and in supraphysiological concentrations, stimulates mitogenesis, probably via non-specific steroid effects. Our observations may have implications for the cardiovascular benefits of low dose estrogens and the risks associated with high dose estrogens seen in epidemiological studies.

1074-125 Proteinase Activated Receptor-2 (PAR-2)-Mediated Mitogenic Responses Are Induced By Human Mast Cell Tryptases

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PAR-2 is the second proteolytically activated receptor that mediates cell activation events by receptor cleavage. PAR-2 is expressed on vascular endothelial cells and is functionally coupled to the thrombin receptor (TR) *in vitro*. To further study previously identified mitogenic effects of PAR-2, we utilized the IL-3-dependent murine lymphoid cell line BaF3 to generate a stable cell line expressing PAR-2 (BaF3/PAR-2). Both wild-type BaF3 and BaF3/PAR-2 cells demonstrated proliferative responses when incubated with! 10% WEHI media (as the source of IL-3), as evaluated by MTT assay. In contrast, only BaF3/PAR-2 cells exhibited mitogenic responses when grown in IL-3-deficient media supplemented with PAR-2 activating peptide (SLIGRL, PAR^{39-44}). No responses were evident in BaF3/PAR-2 cells using the inactive peptide LSIGRL. *Xenopus* oocytes microinjected with PAR-2 cRNA demonstrated a dose-dependent responsiveness to the thrombin receptor activating peptide (TR^{42-47} SFLLRN), and incubation of BaF3/PAR-2 cells with 100 μM TR^{42--47} for 48 hours reproduced the proliferative response, although at ~75% of that identified using equimolar PAR^{39--44}. Because tryptase shares ~70% homology with trypsin (previously shown to activate PAR-2), we evaluated whether expressed forms of α- and β-tryptase could induce proliferative responses in BaF3/PAR-2 cells. Transient transfection of COS-1 cells with human mast cell α- and β-tryptase cDNA's demonstrated tryptase expression in supernatants and cells extracts for both forms, as evaluated by quantitative radioimmunoassay. Comparable proliferative responses were evident using conditioned media from either α- or β-tryptase expressed forms, whereas such responses were limited to α-tryptase cell extracts only. These results identify mast cell tryptases as physiological serine protease agonists for PAR-2 with implications for elucidating the molecular mechanisms regulating cell activation events mediated by proteases generated at the cell-surface during inflammatory, fibrinolytic or thrombosis-regulated pathways.